BCS-based biowaivers: extension to paediatrics

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Abstract

A BCS-based biowaiver allows extrapolation of drug product bioequivalence (when applicable) based on the BCS class of the drug and *in vitro* dissolution testing. Drug permeability and solubility considerations for adult BCS might not apply directly to paediatric subpopulations and bridging of adult and paediatric formulations should be undertaken with caution.

The aims of this study were to: (i.) identify compounds which would change drug solubility classification in the paediatric population, and (ii.) to assess the risk of extending BCS-based biowaiver criteria into paediatric products of these compounds. Amoxicillin, prednisolone, and amlodipine were selected as the model compounds.

Dissolution studies of IR formulations of these compounds were conducted with USP II (paddle) and mini-paddle apparatus, in media of three pHs (pH 1.2, 4.5 and 6.8). Three dissolution setups were tested: (1) 'typical' BCS-based biowaiver conditions, (2) "BE" setup derived from BE study protocols (volume: 250 mL), and (3) "paediatric" setup based on representative volume for the paediatric population (50 mL).

Results revealed that extension of regulated BCS-based biowaiver criteria for paediatric application is not as simple as scaling down volumes. It was further shown that BCS-based biowaiver criteria should not be applied when there is the risk of change of the drug solubility class, from the adult to paediatric populations.

A deeper knowledge of the paediatric gastrointestinal environment is still lacking and would assist in refining the biopharmaceutical tools needed to appropriately evaluate formulation performance across age groups. This would potentially reduce the number of clinical studies required and speed up formulation development.

Keywords: Biopharmaceutics Classification System; Paediatrics; Biowaivers; Dose number; *In vitro* dissolution

Abbreviations

AUC: area under the curve

BA: bioavailability

BCS: biopharmaceutics classification system

BE: bioequivalence

C_{max}: maximum plasma concentration

EMA: European Medicines Agency

FDA: Food and Drug Administration

GI: gastrointestinal

ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IR: immediate release

WHO: World Health Organization

PK: pharmacokinetic

RC: regenerated cellulose

RPM: revolutions per minute

ST: standard deviation

T_{max}: time to maximum concentration

USP: United States Pharmacopeia

USP NF: United States Pharmacopeia and National Formulary

V_0: initial gastric volume available

1. Introduction

Biopharmaceutical tools are extensively used in the design and development of pharmaceutical formulations, namely in risk assessment and optimisation of formulation performance. The application of these tools in paediatric medicines is currently still limited (Batchelor et al., 2013). Despite the increased effort put into improving the safety and effectiveness of paediatric medicines, development of medicines for this population is hindered by ethical considerations and technical constraints (*e.g.* physiological and anatomical changes), leading to knowledge gaps (Batchelor, 2014; Daousani and Karalis, 2017; Elder et al., 2017; Giacoia et al., 2012). Consequently, the tools currently used to undertake biopharmaceutical risk assessment of paediatric formulations are based on adult tests, addressing adult physiology and anatomy (Batchelor et al., 2013). However, the paediatric population has distinct needs with respect to formulation design and performance and thus adult formulations may not be suitable. Due to the challenges faced during paediatric medicine development, regulations have been reformed to address paediatric drug development in parallel to adult formulations (Daousani and Karalis, 2017; Elder et al., 2017). Preliminary *enabling* formulations, *i.e.* drug delivery technologies specially designed to expedite the release and subsequently absorption of drugs, might be used in early paediatric studies, followed by a confirmatory study in which better-designed market formulations are introduced (Ricci, 2013). Supportive clinical studies (*e.g.* relative BA or BE in adults) or *in vitro* techniques may then be used to establish the bridge from adult and/or enabling formulations to the final paediatric formulation.

From a regulatory perspective, during drug development a BE study should be conducted for a new formulation that has not been tested in pivotal efficacy trials. PK parameters of two formulations (*i.e.* AUC, C_{max} and T_{max}) are compared; if the rate and extent of drug absorption fall within predefined limits, comparable *in vivo* drug exposure is ensured. BE studies of

93 paediatric products are currently conducted in adults, with subsequent extrapolation to the
94 target age group and a dose determination/confirmation study (Ricci, 2013).

95 Bioequivalence studies may be exempted if *in vitro* dissolution testing can be used as a
96 surrogate to adequately predict the *in vivo* drug performance (biowaivers), in accordance with
97 regulatory guidelines. The BCS is a scientific tool which categorises drugs according to their
98 (high or low) solubility and intestinal permeability (Amidon et al., 1995). This system has been
99 adopted as a particularly useful tool for *in vivo* drug design and development, particularly in
100 terms of regulatory standards. BCS-based biowaivers have become an important and cost-
101 saving tool in the development of new medicines, formulation bridging and generic drug
102 approval. When combined with *in vitro* dissolution, BCS-based biowaivers take into account
103 the three major factors that govern the rate and extent of oral drug absorption from IR dosage
104 forms. An IR oral solid formulation (test product) is eligible for a BCS-based biowaiver if the
105 drug satisfies solubility criterion (high solubility; BCS class I/III), and the dosage form is
106 pharmaceutically equivalent to the reference product (EMA, 2010; FDA, 2017; ICH, 2018;
107 WHO, 2006). BCS-based biowaiver criteria are detailed in regulatory guidance documents
108 (EMA, 2010; FDA, 2017; ICH, 2018; WHO, 2006). It is required that at least 85 % of the
109 labelled amount of drug substance should dissolve from the product within 15 min (BCS class
110 III drugs) or 30 min (BCS class I drugs), in media across a pH range (pH 1.2, 4.5 and 6.8),
111 using USP apparatus I (100 rpm) or II (50 rpm or 75 rpm (if appropriately justified)). FDA
112 recommends a volume of dissolution media of 500 mL or less (900 mL can be used if
113 appropriately justified); a volume of 900 mL or less is recommended in guidance from the
114 EMA, WHO and in the ICH harmonised draft guideline. The time frame criterion for BCS class
115 I drugs, is subdivided into *very rapidly* and *rapidly* dissolving products (time limits for reaching
116 85% drug dissolved of 15 and 30 min, respectively) and similarity testing (i.e. f_2) is required
117 for rapidly dissolving products. There are no equivalent guidance documents for paediatric

products, and the relevance of the defined criteria in this population is unknown. Overall, the BCS-biowaiver approach has three main requirements: BCS classification, dissolution performance and risk assessment; this risk assessment is used to evaluate the benefits over the risks before a BCS-biowaiver is granted. These requirements would need to be addressed when considering a BCS-based biowaiver for paediatrics.

Currently, biowaiver decisions are based on the drug properties, related to the risk of bioinequivalence in the adult population (based on *e.g.* physiological parameters and prior experience). However, a BCS-biowaiver approach for paediatric products would be beneficial towards producing age-appropriate medicines, whilst minimising/eliminating scientific regulatory risks associated with bioinequivalence in paediatrics. Potentially this could be explored if both the reference (*e.g.* adult formulation or enabling paediatric formulation) and test formulations are pharmaceutical equivalents exhibiting rapid and similar dissolution profiles.

The use of the BCS in paediatrics is limited due to several biopharmaceutical particularities regarding paediatric physiology and PK parameters, therefore BCS-based biowaivers are not feasible for this population (Batchelor, 2014; Batchelor et al., 2016). These particularities include GI pH and volumes, which can influence drug solubility and ionised fraction. Additionally, permeability changes occur as function of the relative size of the small intestine, weight gain and maturation of GI transporters (*e.g.* P-glycoprotein) (Elder et al., 2017; Guimarães et al., 2019). Thus, the role of BCS and biowaivers in paediatric medicine development is unclear (Purohit, 2012; Selen et al., 2010). Additionally, the fact that relative BA and BE studies for paediatric products are performed in adults creates a potential paradox, whereby a drug product would be eligible for a BCS biowaiver based on adult parameters but could fail to meet the high solubility threshold if the volumes were scaled down as appropriate for the paediatric population.

In this context, it is important to investigate the possible changes on the biopharmaceutical characteristics of the drug as a function of the different age groups. Age-related physiological and/or anatomical changes may be responsible for shifts in the BCS classification of a drug due to changes in its solubility and permeability classification (Gandhi et al., 2014). Recent studies have shown that a drug which exhibits a high dose/solubility ratio in adults (*i.e.* highly soluble drugs) might not show the same ratio in paediatric patients, and unfavourably shift into poorly soluble classification. Consequently, these drugs would not be eligible for BCS-based biowaivers in the pediatric populations (delMoral-Sanchez et al., 2019; Gandhi et al., 2014; Shawahna, 2016).

The aims of this study were to assess the risk of extending the biowaiver criteria for IR formulations from adults to paediatrics, and to identify bioinequivalence risks when comparing the performance of different formulations in age-appropriate BCS-conditions. The biowaiver decision was then discussed not only in terms of the formal requirements set out in existing guidance, but also in the context of the risks associated with an incorrect biowaiver decision. Drugs were selected based on the identified risk of shifting into poorly soluble classification in the different paediatric age groups and, consequently, not being eligible for a BCS-based biowaiver. Amoxicillin, amlodipine and prednisolone were selected as the model compounds.

2. Materials and methods

2.1 Materials

Sodium hydroxide, 37 % hydrochloric acid, sodium chloride, glacial acetic acid, potassium dihydrogen phosphate, sodium acetate trihydrate and sodium phosphate anhydrous were purchased from Fisher Scientific (UK). Water was ultra-pure (Milli-Q) laboratory grade. RC membrane filters (0.45 µm) were from Cronus® (UK). Amoxicillin trihydrate (98 %) was

purchased from VWR (UK). Prednisolone (99 %) and amlodipine besylate (pharmaceutical secondary standard) were obtained from Sigma-Aldrich (UK). Details of the formulations used are presented in Table 1.

Table 1 here

2.2 Methods

2.2.1 Drug and dose selection

Amoxicillin (trihydrate), prednisolone and amlodipine (besylate) were selected as the model compounds. They are included in the Model List of Essential Medicines for Children, with two dose strengths specified for each drug (WHO, 2017). Two doses (a 'low' dose for paediatrics and a 'high' dose for adults) were selected for this study; these were: 250 and 500 mg for amoxicillin, 5 and 25 mg for prednisolone and 5 and 10 mg for amlodipine.

The BCS allows the classification of drugs as highly soluble when the highest drug dose (or dose unit, D_0) is soluble in 250 mL of an aqueous liquid, at a relevant physiological pH range of 1.2 – 6.8 (Amidon et al., 1995). According to this criterion, the three drugs (all doses used in this study) selected are classified as highly soluble drugs (Shohin et al., 2010; Thambavita et al., 2017; Vogt et al., 2007). The key factors that define drug dose unit (*i.e.* highest dose strength, initial gastric volume available (V_0) and drug solubility) have been shown to vary amongst the different populations (Abdel-Rahman et al., 2012; Gandhi et al., 2014; Shawahna, 2016). The paediatric D_0 of these drugs were estimated across the different paediatric age groups (average age in each subpopulation was used for the calculations; Table 2), using Equation 1:

$$D_0 = \frac{\text{(Paediatric dose)}}{V_0 \times \text{Drug solubility}} \qquad \text{(Eq. 1)}$$

where, aqueous drug solubility data was obtained from the literature (Wishart et al., 2007), and the initial gastric volumes for the paediatric subgroups were determined using Equation 2:

$$V_0 = \frac{\text{Weight (kg)}\ x\ 0.56\ \text{(mL/kg)}}{37.1\ \text{(mL)}}\ x\ 250\ \text{mL} \qquad \text{(Eq. 2)}$$

where, 0.56 mL/kg and 37.1 mL are estimates of fasted gastric fluids volumes in paediatrics (Crawford et al., 1990) and adults (Goetze et al., 2009), respectively, and 250 mL is the reference volume used in the BCS (EMA, 2010; FDA, 2017; WHO, 2006).

All drugs were shown to change D_0 (Table 2) and consequently BCS class, with a shift from high drug solubility classification in adults to low drug solubility classification (given by $D_0 >$ 1 (Batchelor, 2014)) in certain paediatric age groups. The BCS-based biowaiver status claimed in adults may therefore not be safely extended to the paediatric population. Therefore, these drugs were selected as the model compounds for this study.

Table 2 here

2.2.2 *In vitro* dissolution studies

In vitro dissolution studies were conducted with USP II apparatus or mini-paddle apparatus (Agilent 708-DS Dissolution apparatus; Agilent, USA). For the mini-paddle setup, TruAlign 200 mL vessels and electropolished stainless steel mini-paddles were used (Agilent, USA). Experiments were conducted at 37 ± 0.5ºC in three media; simulated gastric fluid *sine pepsin* (SGF*sp*) pH 1.2, acetate buffer pH 4.5 and phosphate buffer pH 6.8 (USP, 2007). Three different setups were developed for the assessment of formulation performance and

equivalence, in dissolution conditions representative of both adults and paediatric populations (Figure 1). Setup 1 was conducted in USP II apparatus, using 900 mL of dissolution media (maximum volume recommended in regulatory guidance documents) and an agitation of 50 rpm (prednisolone and amlodipine) or 75 rpm (amoxicillin) (setup 1, 'typical' BCS-based biowaiver conditions). Setup 2 was conducted in USP II apparatus, using 250 mL of media and an agitation rate of 50 rpm (prednisolone and amlodipine) or 75 rpm (amoxicillin) (setup 2, derived from BE study protocols that prescribe administration of a drug product to fasting human volunteers with a glass of water of 250 mL). Setup 3 was conducted in mini-paddle apparatus, using 50 mL of dissolution media and an agitation rate of 125 rpm (prednisolone and amlodipine) or 187.5 rpm (amoxicillin) (setup 3, where a 50 mL volume representative of the paediatric population was used). The agitation rate for setup 3 in the mini-paddle apparatus (125 or 187.5 rpm) was set based on the speed factor of 2.5 between paddle and mini-paddle hydrodynamics [*i.e.* Agitation rate mini-paddle = 2.5 * agitation rate paddle] to reflect the agitation rate used in the USP II apparatus (50 or 75 rpm, respectively) (Scheubel et al., 2010). Other requirements for granting the biowaiver status (*i.e.* pH range for testing and time frame limits for rapid dissolution of the formulations) were maintained for all setups, as per current regulations.

For amoxicillin capsules, slow dissolution was observed when testing at 50 rpm in media of pH 4.5 and 6.8. To explore the dissolution performance of the drug products and investigate if there were experimental issues of coning, dissolution tests were performed with Amoxil® 250 and 500 mg capsules (reference product), in media of pH 4.5 and 6.8 at three agitation rate conditions; 50, 75 and 100 rpm. Two volumes were tested: 900 mL (setup 1) and 250 mL (setup 2).

Experiments were conducted for 60 min or 120 min, depending on whether complete dissolution was reached within 60 min. For amoxicillin testing, capsules were put in sinkers

(Quality Lab Accessories LCC, USA). Sample collection took place at 5, 10, 15, 20, 30, 45, 60, 75, 90 and 120 min. 2 mL samples were withdrawn (with volume replacement) using a 2 mL glass syringe (Fortuna Optima® fitted with a stainless tubing) through a cannula (Quality Lab Accessories LCC, USA). Each sample was filtered with a RC filter (0.45 µm), and appropriately diluted prior to quantitative analysis. All experiments were performed in triplicate. Samples were analysed at 272 (amoxicillin), 246 (prednisolone) and 239 (amlodipine) nm, using an UV spectrophotometer (Thermo Scientific Helios Gamma UV-Vis Spectrophotometer, Thermo Fisher Scientific, UK) equipped with a cuvette.

Drug quantification was performed based on calibration curves prepared in the corresponding media for each experiment. Freshly prepared standard solutions (concentration range: 5 – 120 µg/mL (amoxicillin) or 2 – 30 µg/mL (prednisolone and amlodipine)) were prepared by appropriate dilution of a 1000 µg/mL stock solution of the analytical standard in water (amoxicillin) or methanol (prednisolone and amlodipine). The interfering effect of formulation excipients on the maximum absorption of the compounds was deemed insignificant, after scanning and comparing the spectrum of each stock solution with the spectrum of a solution of same concentration of the dissolved dosage forms in water (amoxicillin) or methanol (prednisolone or amlodipine) (data not shown). Results were expressed as mean percentage (%) drug dissolved ± S.D., at the given sampling time.

Figure 1 here

2.2.3 Treatment of dissolution data

To qualify for a BCS-based biowaiver, both the test product and reference should display a mean % drug dissolved above 85 % within 15 or 30 min, and similar *in vitro* dissolution

characteristics, under all the defined conditions (*i.e.* agitation rate, pH range). When > 85% of the label amount of drug was dissolved in 15 min (for both test and reference products), the dissolution profiles were considered similar. If this was not the case, the similarity factor f_2 was estimated for comparison of dissolution profiles, by using the following equation (Eq. 3) (Shah et al., 1998):

$$f_2 = 50 \bullet \log\left(\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{n}[\overline{R}(t)-\overline{T}(t)]^2}{n}}}\right) \qquad \text{(Eq. 3)}$$

where, n is the number of time points, $\overline{R}(t)$ is the mean percent of reference drug dissolved at time t after starting the study; and $\overline{T}(t)$ is the mean percent of test drug dissolved at time t.

DDsolver® software (an Add-In for Excel, Microsoft®) was used to calculate the similarity factor f_2. The coefficient of variation was less than 20% at early time points and less than 10% at other time points, allowing the use of mean values for the calculation of the similarity factor; only one measurement was considered after 85% dissolution of each product (FDA, 2017). Two dissolution profiles were considered similar when the f_2 value was ≥ 50 (Shah et al., 1998).

3. Results and discussion

3.1 Amoxicillin

Amoxicillin can be classified as a BCS class I drug, for doses under 875 mg, according to drug solubility and permeability studies (Thambavita et al., 2017). According to regulations for BCS-based biowaivers, dissolution studies should be performed with USP II paddle apparatus at 50 rpm. Amoxicillin capsules had a slow and incomplete dissolution when dissolution testing was performed at this agitation rate in media of pH 4.5 and 6.8. At an agitation rate of 75 rpm and 100 rpm, dissolution of amoxicillin from the Amoxil® capsules (reference product; 250 mg

and 500 mg) was rapid and reached completion, with low variability between replicates. These results revealed that at lower agitation rate (50 rpm) coning effect was taking place (Figure 2). Therefore, the dissolution tests for the amoxicillin capsules were performed at 75 rpm (setup 1 and 2) and 187.5 rpm (setup 3).

Figure 2 here

Dissolution of amoxicillin from the formulations tested (reference: Amoxil®; test: Teva® and Kent®) is presented in Figure 3 and f_2 similarity factors estimated for comparison of the dissolution profiles in Table 3.

For the 250 mg amoxicillin capsules, under setup 1 conditions at pH 1.2, more than 85% of amoxicillin was dissolved from the tested formulations within 15 min. In the acetate (pH 4.5) and phosphate (pH 6.8) buffers, dissolution of all the tested products was not rapid (% drug dissolved within 30 min was less than 85%). Dissolution of amoxicillin was complete within 15 min in pH 1.2 media, 90 min in pH 4.5 media and 45 min in pH 6.8 media, and the dissolution profiles of the tested products (Teva® and Kent®) were similar to the dissolution profile of the reference product (Amoxil®) ($f_2 \geq 50$; Table 3). Consequently, the products would not qualify for biowaiver status. These results are in agreement with dissolution studies recently conducted based on USP methodologies and BCS-based biowaiver dissolution studies, which have showed high failure rates for amoxicillin products (Löbenberg et al., 2012; Reddy et al., 2014; Stuart et al., 2014). The discrepant dissolution profiles are likely caused by poor manufacturing techniques or variation in the API particle size, and thus with appropriate content uniformity assays in addition to *in vitro* drug dissolution testing, this risk should be easily identified. Under setup 2 conditions, more than 85% of the labelled amount of

amoxicillin was dissolved in less than 15 min at pH 1.2, and under 30 min at pH 4.5 and 6.8 for all products tested. Complete dissolution was achieved within 20 min in pH 1.2 media, and 45 min in pH 4.5 and 6.8 media. Similarity comparison of the Teva® and Kent® products with the reference product showed that biowaiver status would be granted (pH 1.2: all products *rapidly* dissolved; pH 4.5: f_2 = 60.6 (Amoxil®-Kent®) and 62.8 (Amoxil®-Teva®); pH 6.8: f_2 = 73.1 (Amoxil®-Kent®) and 61.0 (Amoxil®-Teva®)). Since *in vitro* equivalence was shown between Amoxil® and the test products, the amoxicillin Teva® and Kent® capsules would be assumed as therapeutically equivalent to the reference product, under these testing conditions. With setup 3 conditions, the criterion for rapid dissolution was not met within the pH range tested. Dissolution was complete at pH 1.2 and 6.8 (within 15 and 75 min, respectively), but not at pH 4.5. Therefore, even though the products were shown to be similar ($f_2 \geq 50$; Table 3), they would not qualify for biowaiver status. The different results obtained with setups 1 and 3 in comparison to setup 2 testing conditions may be related to the dissolution setup. When using a dissolution volume of 250 mL in USP II dissolution (setup 2), the paddles are very close to the medium surface, which not only requires careful sampling as it may lead to result variability but also shows that the different hydrodynamics impact drug dissolution, ultimately affecting the outcome of the product qualification for a BCS-based biowaiver.

For the 500 mg capsules, the amoxicillin products would not qualify for a BCS-biowaiver under any of the setup conditions tested. Even though similarity was shown between the test and reference products ($f_2 \geq 50$; Table 3), the criterion for rapid dissolution was not met in any of the setups tested (setup 1 to 3; pH 1.2: all products *rapidly* dissolved; pH 4.5: % drug dissolved within 30 min was less than 85%; pH 6.8: drug dissolved within 30 min was less than 85% under setup 2 and 3 conditions). Under setup 1 conditions, complete dissolution was achieved in all media pH (100% drug dissolved reached within 15, 90 and 45 min at pH 1.2, 4.5 and 6.8, respectively). Under setup 2 conditions, complete dissolution was achieved in pH 1.2 and 6.8

media within 10 and 90 min, respectively, but not in pH 4.5 media. Under setup 3 conditions, complete dissolution was only achieved in pH 1.2 media, at pH 4.5 and 6.8 the maximum % dissolved within 2 h was 60 and 80%, respectively. These results show a clear impact of the pH-drug solubility profile on drug dissolution behaviour. Amoxicillin is an amphoteric compound (Thambavita et al., 2017); in acidic pH it is protonated, in a pH typical of the upper small intestine it exists primarily as a zwitterion, and in the distal small intestine (pH 6.5) it will exist both as zwitterion and as deprotonated acid. It has been shown to exhibit a pH-dependent, *U*-shaped solubility curve (drug solubility in buffers of pH 1.2, 4.5, and 6.8 was 7.7, 3.6 and 5.4 mg/mL, respectively) (Thambavita et al., 2017). Accordingly, drug dissolution rate was higher at pH 1.2, which can be correlated with the higher drug solubility in acidic conditions due to an increase in the ionisation % of the drug. For the 250 mg products (setup 3) and 500 mg products (all setups), sink conditions were not achieved during the dissolution studies (*i.e.* having a volume of medium at least three times above the volume required to form a saturated drug solution (Gibaldi and Feldman, 1967)). Sink conditions are critical to ensure that reproducible dissolution occurs; moreover, under non-sink conditions *in vitro* results may have little relationship with *in vivo* observations (Gibaldi and Feldman, 1967).

Overall, the 250 mg amoxicillin products tested would fail to meet the *in vitro* dissolution criterion associated with the BCS-based biowaiver requirements in setups 1 and 3 conditions and would pass in setup 2 conditions. As previously mentioned, the difference in outcomes between setup 2 conditions and the other setups is likely related to the impact of hydrodynamics. For the 500 mg amoxicillin capsules, the tested products would fail to meet BCS-based biowaiver requirements in all the setup scenarios tested.

Amoxicillin is a broad spectrum, beta-lactam antibiotic, mainly used in an ambulatory setting for infections of mild-to-moderate severity (Eyer, 2002; Thambavita et al., 2017). Since it has a wide therapeutic range (Doogue and Polasek, 2011), the possibility of life-threatening toxic

358 reactions with supra-therapeutic doses of amoxicillin is very low. On the other hand, the risk
359 associated with subtherapeutic blood levels is unknown; a false-positive biowaiver decision,
360 particularly if the products are severely below the accepted level of bioequivalence, could
361 possibly lead to prolongation of illness, and even to the development of resistance if the drug
362 content significantly differs from the labelled amount (Gustafsson et al., 2001). In this study,
363 the products are not rapidly dissolved in pH 4.5 in setup 1 conditions and a biowaiver for an
364 adult formulation would not be granted. Furthermore, since the paediatric population undergoes
365 developmental changes (*e.g.* gastric pH and emptying, intestinal transit time, membrane
366 permeability, body water, distribution and metabolism), which may lead to a significant
367 alteration of the plasma concentration profile and of key bioequivalence parameters (*e.g.* C_{max}
368 and AUC), bioinequivalence risks might be increased in this population (Batchelor et al., 2014;
369 Guimarães et al., 2019). Consequently, a BCS-based biowaiver status of these products could
370 also not be applied for administration in paediatrics.

371

372 *Figure 3 and Table 3 here*

373

374 **3.2 Prednisolone**

375 Dissolution profiles of prednisolone from the products tested (reference: Pevanti®; test:
376 Actavis®) are presented in Figure 4 and f_2 similarity factor results are shown in Table 3.

377 Results of dissolution studies of the 5 mg prednisolone tablets revealed that more than 85% of
378 the labelled amount of prednisolone was dissolved in less than 15 min, under all the setup
379 scenarios tested. Under setup 1 and 2 conditions, complete dissolution was achieved in pH 1.2
380 and 6.8 media within 15 min and in pH 4.5 media within 20 min. Under setup 3 conditions,
381 complete dissolution was achieved in all pH within 30 min. Due to having met *very rapidly*

dissolution criterion, *in vitro* equivalence was shown between prednisolone Actavis® and Pevanti® tablets, in all the setup testing conditions performed. The test product can be assumed as therapeutically equivalent to the reference, with no need for *in vivo* bioequivalence studies.

For the 25 mg tablets, under setup 1 conditions, complete dissolution was achieved in pH 1.2 media within 20 min and in pH 4.5 and 6.8 media within 45 min. Comparison of the dissolution studies of the test (Actavis®) and the reference (Pevanti®) products showed that following BCS-based dissolution testing conditions, biowaiver status would be granted (*rapidly* dissolved products; $f_2 \geq 50$; Table 3). Under setups 2 and 3 dissolution conditions, the products would not qualify for biowaiver status. Under setup 2 dissolution conditions, although more than 85% of the labelled drug amount in the dosage form was dissolved in less than 30 min, f_2 analysis revealed that the test and reference products were not similar (pH 4.5 and 6.8: f_2 (Actavis®-Pevanti®) = 47.5 and 38.0, respectively). Under these conditions, complete dissolution was achieved within 20 min in pH 1.2 media and within 45 min in pH 4.5 and 6.8 media. When testing under setup 3 dissolution conditions (mini-paddle apparatus, 125 rpm, 50 mL), the test product would not qualify for BCS-based biowaiver status since rapid dissolution criterion (> 85% dissolved in less than 30 min) was not met, even though f_2 analysis revealed similarity between test and reference products ($f_2 > 50$; Table 3). Sink conditions in dissolution testing of both products were not achieved with this dose (25 mg) in 50 mL, as revealed by the lower dissolution (maximum % drug dissolved at 2 h was 89.0 and 85.5%, for Pevanti® and Actavis®, respectively).

Overall, the 5 mg prednisolone products tested would meet the *in vitro* dissolution criterion associated with the BCS-based biowaiver requirements, in all the setup conditions tested. For the 25 mg tablets, the products would meet BCS-based biowaiver requirements in setup 1 conditions, but not under setup 2 and 3 conditions tested.

According to drug solubility and permeability studies, prednisolone can be classified as a BCS Class I drug (Vogt et al., 2007). When calculating the D_0 for the 25 mg tablets, prednisolone was shown to change solubility class to low solubility in younger age groups (Table 2). This is reflected in the results obtained when testing the 25 mg formulations, under setup 2 and 3 conditions. In this context, formulation bridging into paediatrics could result in a false biowaiver decision (*i.e.* wrongly declaring the test formulation bioequivalent to the reference formulation in the paediatric population). This could affect the *in vivo* drug behaviour, resulting in changes in the AUC and/or C_{max} of the drug. If resulting in a lower AUC, the products might be clinically less effective in paediatric patients and/or potentially lead to serious clinical consequences when acute treatment is required for severe, life-threatening diseases. In this case, as prednisolone is a prescription-only drug, therapy should be periodically reviewed, and a dose adjustment/ substitution would be required. If the drug became supra-bioavailable (*i.e.* resulted in a higher AUC than intended), the risk of toxicity and/or side effects would increase (Robinson et al., 2016; Vogt et al., 2007). If bioinequivalence was caused by a difference in C_{max}, clinical implications could be expected since prednisolone IR tablets are usually used in chronic therapeutic regimes (Paediatric Formulary Committee, 2017).

Figure 4 here

3.3 Amlodipine

Dissolution of amlodipine from the products tested (reference: Istin®; test: Sandoz® and Teva®) and f_2 similarity factor results are presented in Figure 5 and Table 3, respectively.

For the 5 mg tablets, under setup 1 testing conditions, more than 85% of amlodipine was dissolved within 15 min, in pH 1.2 and pH 4.5 media. At pH 6.8, although f_2 analysis revealed

similarity between the dissolution profiles of the tested products in relation to the reference, the criterion for rapid dissolution was not met (*i.e.* 85% of drug dissolution within 30 min). Therefore, the products would not qualify for biowaiver status. Complete dissolution was achieved in pH 1.2 and 4.5 media within 10 min and in pH 6.8 media within 60 min. The lower dissolution rate observed at pH 6.8 could be explained by drug characteristics; since amlodipine is a weak base (pKa 8.7 (Van Zwieten, 1994)), it is affected by changes in pH and exhibits pH-dependent solubility (Shohin et al., 2010; Van Zwieten, 1994). Under setups 2 and 3 conditions, more than 85% of the labelled amount of amlodipine was dissolved in less than 15 min. Having met the *very rapidly* dissolution criterion, *in vitro* equivalence was shown between the test products and the reference, and the products would qualify for BCS-biowaiver status.

For the 10 mg tablets, under setup 1 (BCS-based dissolution testing) and setup 2 conditions, % drug dissolved was higher than 85% under 15 min at pH 1.2 and 4.5, and under 30 min at pH 6.8, for all products tested. Under both setup conditions, complete dissolution was achieved within 15 min in media of pH 1.2 and 4.5, and within 45 (setup 1) or 60 (setup 2) min at pH 6.8. Similarity comparison of the Teva® and Istin® (reference) tablets showed that biowaiver status would be granted for the test product (pH 1.2 and 4.5: *rapidly* dissolved products; pH 6.8: f_2 (Istin®-Teva®) = 50.7 and 64.3 for setups 1 and 2, respectively). On the contrary, comparison of Sandoz® and reference (Istin®) tablets showed that biowaiver status would not be granted for the test product (pH 1.2 and 4.5: *rapidly* dissolved products; pH 6.8: f_2 (Istin®-Sandoz®) = 48.4 and 40.0 for setups 1 and 2, respectively). In the case of the formulations tested (10 mg tablets), the differences observed in the dissolution of amlodipine could be attributed to the excipients used and/or manufacturing methods. Under setup 3 conditions (mini-paddle, 50 mL, 125 rpm), complete dissolution was achieved in pH 1.2 and 4.5 media within 10 min and in pH 6.8 media within 45 min. % drug dissolved was higher than 85% under 15 min at pH 1.2 and 4.5, and under 30 min at pH 6.8 for all products tested, and f_2

455 analysis revealed similarity between all products (pH 1.2 and 4.5: *rapidly* dissolved products;
456 pH 6.8: f_2 (Istin®-Sandoz®) = 81.4, f_2 (Istin®-Teva® = 52.7)). Therefore, biowaiver status would
457 be granted for all products.

458 Overall, results revealed that the 5 mg amlodipine tablets tested would fail to meet the *in vitro*
459 dissolution criterion associated with the BCS-based biowaiver requirements in setup 1
460 conditions and would pass in setup 2 and 3 conditions. Regarding the 10 mg amlodipine tablets,
461 the Istin® and Teva® products would qualify for a BCS-based biowaiver status under all setup
462 scenarios tested, while the Sandoz® products would not qualify for a biowaiver status in setup
463 1 and 2 conditions but would qualify in setup 3 conditions.

464 Regarding evaluating patient risks associated with bioinequivalence, a false-positive biowaiver
465 decision for amlodipine IR dosage forms could result in subtherapeutic plasma concentrations
466 (which may lead to a therapeutic failure) or to concentrations above the recommended upper
467 therapeutic concentrations (which may result in adverse drug reactions). Amlodipine is
468 indicated for hypertension (Flynn et al., 2000; Murdoch and Heel, 1991; Van Zwieten, 1994).
469 In general, drug dose is individualized depending on the severity of disease, tolerance and
470 responsiveness of the patient to the drug (Murdoch and Heel, 1991). In these situations, it is
471 necessary to ensure BE of the product, so that the therapeutic outcome from treatment with test
472 products could be well predicted during the management of pharmacological indications. As
473 far as supra-therapeutic drug levels, mild to moderate side effects have been reported (Murdoch
474 and Heel, 1991). Patient risks associated with the subtherapeutic levels pose more serious
475 consequences because of therapeutic insufficiency; these can be exacerbated in very young age
476 groups as a recent study has shown that amlodipine dosing has a significant inverse relationship
477 with patient age, with the youngest children requiring the highest doses of amlodipine (Flynn
478 et al., 2000).

Figure 5 here

3.4 Risk assessment of extending BCS-based biowaiver criteria into paediatrics

A summary of the results obtained in this study, and assessment of whether the biowaiver status as currently defined would be granted in each situation, is presented in Figure 6.

Results revealed that only the 5 mg prednisolone and the 10 mg amlodipine tablets (Istin® and Teva® but not Sandoz®) would qualify for a biowaiver status, under all setup scenarios tested. In view of these results, it seems clear that extrapolation of the BCS-based biowaiver criteria into paediatrics is not straightforward and cannot be based on direct assumptions (*i.e.* simple scaling down).

When analysing the risk of extending BCS-based biowaiver testing criteria into products for the paediatric population, it is important to consider the factors that would affect BCS-based biowaiver decisions and the relevance of the criteria within the paediatric population. BCS-based biowaiver decisions are considered for highly soluble drugs, which are expected to exhibit fast dissolution rates. Currently, drugs are classified as highly soluble if the highest dose strength is soluble in at least 250 mL of aqueous liquid at a relevant physiological pH range of 1.2 – 6.8, however these aspects concern adult physiology. Age-related changes in anatomy and physiology will impact the classification of drug solubility and permeability properties within the different paediatric subpopulations. Several issues arise regarding drug solubility classification amongst the paediatric population, including the definition of the highest single dose, the initial gastric volume, and the luminal solubility of the drug. Moreover, with respect to drug permeability classification, drugs are classified as highly permeable when the extent of oral absorption (*i.e.* fraction of dose absorbed) is greater than 85% of the

503 administered dose. However, using adult permeability data for paediatric subjects is
504 controversial and information regarding permeability in younger paediatric subgroups is still
505 lacking, hindering the establishment of meaningful permeability criteria for this population.
506 Similarly, a drug that exhibits a high dose/solubility ratio in adults (*i.e.* highly soluble drug)
507 might not show the same ratio in paediatric patients, and unfavourably shift into poorly soluble
508 classification. In this study, except for the case of prednisolone in a dose of 5 mg, the chosen
509 model compounds selected would change from high drug solubility classification in adults to
510 low drug solubility class in paediatric age groups ($D_0 > 1$; Table 2). These drugs would not be
511 eligible for BCS-based biowaiver status, as the solubility criterion was not met, even at the
512 relevant paediatric doses tested. In this context, a p-BCS approach could provide a simplistic
513 tool to help understand possible age-related physiological and/or anatomical changes in oral
514 drug performance, and identify risks associated with a change in BCS class of a compound and
515 eligibility for BCS-based biowaivers for paediatric products.

516 Regarding testing methodology, dissolution testing with USP I/II apparatus is commonly used
517 to assess the dissolution rate of drug products, and in the case of BCS-based biowaivers rapid
518 dissolution is required across a pH range. The basis of this approach is that the dosage form is
519 agitated at a fixed rate within a fixed media volume, representative of the GI environment.
520 Some limitations associated with this apparatus have been reported, including the impossibility
521 of using small testing volumes (Crist, 2009; Scheubel et al., 2010). This is of importance as the
522 fluid volumes available in the GI tract of younger age groups are smaller than adults (Batchelor
523 et al., 2014). In the present study, an adaptation of standard USP II apparatus to a mini-paddle
524 apparatus was tested as an appropriate method to address the need for small volume testing.
525 The mini-paddle apparatus is already commonly used when screening for critical quality
526 attributes of rapid dissolving tablets, in cases where it is difficult to detect differences using
527 standard working conditions (Crist, 2009). Regarding agitation rate criterion, rates of 50 and

100 rpm have been defined for paddle and basket apparatus, respectively (Batchelor et al., 2013; FDA, 2017; Purohit, 2012). A direct extrapolation from the agitation rates set for the USP II apparatus (50 or 75 rpm) to the mini-paddle was made according to a speed factor, which allowed the maintenance of discriminatory conditions.

The dissolution media volume was considered in this study and its effect on drug dissolution was evaluated by comparison of different setup conditions. Volume scaling down is a commonly used approach in paediatric biopharmaceutics to simulate the lower gastrointestinal volumes of the paediatric population in comparison to adults. Since currently there is no guidance on appropriate volumes to use in paediatric dissolution testing, a direct extrapolation from the adult value of 250 mL utilised in USP II dissolution was conducted for the mini-paddle apparatus (50 mL; setup 3). An important factor in BCS-style bridging is that dissolution rate of paediatric medicines needs to be rapid to ensure adequate exposure in this population and guarantee that GI transit dictates drug absorption rather that drug dissolution. In this context, the 5 mg prednisolone and 10 mg amlodipine tablets (Istin® and Teva® but not Sandoz®) would be eligible for a BCS-based biowaiver; however, all the amoxicillin products (250 and 500 mg), the lowest dose amlodipine products and the highest dose products of prednisolone would fail to be classified as *rapidly* dissolved. In the case of the amlodipine 5 mg tablets, the products would fail the criterion of rapid dissolution under setup 1 conditions (current requirements for BCS-based biowaivers) due to slow dissolution in pH 6.8 but would pass when the volume was scaled down (setups 2 and 3). In the case of the prednisolone 25 mg tablets, the products would meet the criterion of rapid dissolution under setup 1 conditions (current requirements for BCS-based biowaivers), but would fail when the volume was scaled down, likely due to the solubility of the drug.

The time limits set to define rapid dissolution criteria might also affect the biowaiver status, as subsequent analysis of the dissolution profiles differs accordingly. For example, in the present

study it was shown that when the products did not meet the criteria for *very rapidly* or *rapidly* dissolving products, it was because both the test and reference products did not exhibit fast dissolution and not due to dissimilarity between profiles (except for the case of the Sandoz® amlodipine 10 mg tablets). This could indicate that the time frame for rapid drug dissolution should be further evaluated, and potentially refined when considering the paediatric population. A minimum of 50% of drug release within 15 min has been recently suggested to support a biowaiver decision for paediatric formulations (Abdel-Rahman et al., 2012). With this criterion, the formulations studied in mini-paddle setup would be considered as *rapidly* dissolved, except for the case of amoxicillin 500 mg capsules for which drug dissolution was shown to be limited by drug solubility. The scientific basis for such alterations regarding the most appropriate time frames for evaluating dissolution rates, would need to be further evaluated.

Excipients have also been shown to affect the fraction of dose absorbed (*e.g.* by modulating disintegration, drug solubilisation or stabilizing a specific polymorphic form), and therefore might impact the drug dissolution characteristics (Batchelor et al., 2014). This impact may be more significant in very young paediatric groups, for whom certain excipients might affect GI absorption, even if they have been considered safe and acceptable for adults (Batchelor et al., 2014; Walsh et al. 2014).

Another point to consider is that tablets are commonly crushed and sometimes mixed with food/drinks prior to paediatric administration, which might affect drug solubilization and dissolution behaviour (Martir et al., 2020). These potential changes were not considered in this study as the aim was to study the extrapolation of the current protocol used for BCS-based biowaivers to paediatrics.

Overall, the risk of using the BCS adult classification in paediatric drug development lies in shifts in BCS classification of drugs due to growth and maturation of paediatric subpopulations. Results from this study reveal the need for the development and establishment of a p-BCS, as a simplistic tool to help understand possible changes in oral drug performance in the paediatric population. The development and establishment of a p-BCS could meaningfully impact the paediatric biopharmaceutical field and guide the production of age-appropriate medicines and facilitate formulation bridging. While such a tool remains to be developed, extrapolation of the adult BCS classification should be performed with care.

Figure 6 here

4. Conclusions

The use of BCS-based biowaivers for paediatric products needs to be undertaken with caution due to differences in the drug D_0 between adults and paediatrics.

In this study, the risk of directly extrapolating BCS-based criteria into paediatrics was assessed. A dissolution setup potentially representative of the paediatric population in terms of the lower volumes required was tested (setup 3), with the criteria limits used in BCS-based biowaiver guidance being applied for product evaluation/risk assessment. It was shown that a simple scaling down of the dissolution testing volume stipulated on BCS-based biowaiver dissolution criteria may not be adequate for paediatric products. Knowledge of the solubility classification of a drug across different age groups, would assist on assessing the development of a biowaiver as BE testing surrogate in the different age groups. Therefore, a consensus on a p-BCS needs to be reached and should address the heterogeneity of the paediatric population.

Overall, the establishment of a p-BCS would contribute to formulation bridging (*e.g.* surrogate the need for future clinical BE testing) and risk assessment decisions, thus promoting paediatric drug development. This would result in a smaller discrepancy between technologies available for the different age groups and provide better support for the development and testing of age-appropriate medicines, ultimately leading to a minimisation of clinical trials and regulatory burden.

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References

Abdel-Rahman, S.M., Amidon, G.L., Kaul, A., Lukacova, V., Vinks, A.A., Knipp, G.T., Force, B.T., 2012. Summary of the national institute of child health and human development–best pharmaceuticals for children act pediatric formulation initiatives workshop–pediatric biopharmaceutics classification system working group. Clin Ther 34, S11-S24. https://doi.org/10.1016/j.clinthera.2012.09.014.

Amidon, G.L., Lennernäs, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res. 12, 413-420. https://doi.org/10.1023/a:1016212804288.

Batchelor, H., 2014. Paediatric biopharmaceutics classification system: current status and future decisions. Int J Pharm 469, 251-253. https://doi.org/10.1016/j.ijpharm.2014.02.046.

Batchelor, H., Ernest, T., Flanagan, T., Klein, S., Turner, R., Fotaki, N., Storey, D., 2016. Towards the development of a paediatric biopharmaceutics classification system: results of a survey of experts. Int J Pharm 511, 1151-1157. https://doi.org/10.1016/j.ijpharm.2016.

Batchelor, H.K., Fotaki, N., Klein, S., 2014. Paediatric oral biopharmaceutics: key considerations and current challenges. Adv Drug Deliv Rev 73, 102-126. https://doi.org/10.1016/j.addr.2013.10.006.

Batchelor, H.K., Kendall, R., Desset-Brethes, S., Alex, R., Ernest, T.B., Initiative, E.P.F., 2013. Application of in vitro biopharmaceutical methods in development of immediate release oral dosage forms intended for paediatric patients. Eur J Pharm Biopharm 85, 833-842. https://doi.org/10.1016/j.ejpb.2013.04.015.

Crawford, M., Lerman, J., Christensen, S., Farrow-Gillespie, A., 1990. Effects of duration of fasting on gastric fluid pH and volume in healthy children. Anesth Analg 71, 400-403. https://doi.org/10.1213/00000539-199010000-00014.

Crist, G.B., 2009. 2009 Trends in Small-Volume Dissolution Apparatus for Low-Dose Compounds. Dissolut Technol 16, 19-22. https://doi.org/10.14227/DT160109P19.

Daousani, C., Karalis, V.D., 2017. Paediatric medicines: regulatory and scientific issues. Drug Res. 67, 377-384. https://doi.org/10.1055/s-0043-109788.

delMoral-Sanchez, J.M., Gonzalez-Alvarez, I., Gonzalez-Alvarez, M., Navarro, A., Bermejo, M., 2019. Classification of WHO Essential Oral Medicines for Children Applying a Provisional Pediatric Biopharmaceutics Classification System. Pharmaceutics 11. http://doi.org/10.3390/pharmaceutics11110567.

Doogue, M.P., Polasek, T.M., 2011. Drug dosing in renal disease. Clin Biochem Rev 32, 69.

Elder, D.P., Holm, R., Kuentz, M., 2017. Medicines for pediatric patients—biopharmaceutical, developmental, and regulatory considerations. J Pharm Sci 106, 950-960. https://doi.org/10.1016/j.xphs.2016.12.018.

EMA, 2010. Committee for Medicinal Products for Human Use: Guideline on the investigation of bioequivalence. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf (accessed 5 January 2019),

Eyer, C.L., 2002. Goodman & Gilman's: The Pharmacological Basis of Therapeutics. Am J Pharm Educ 66, 95

FDA, 2017. Guidance for industry: waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. https://www.fda.gov/downloads/Drugs/Guidances/ucm070246.pdf (accessed 5 January 2019),

Flynn, J.T., Smoyer, W.E., Bunchman, T.E., 2000. Treatment of hypertensive children with amlodipine. Am J Hypertens 13, 1061-1066. https://doi.org/10.1016/s0895-7061(00)00278-8.

Gandhi, S.V., Rodriguez, W., Khan, M., Polli, J.E., 2014. Considerations for a Pediatric Biopharmaceutics Classification System (BCS): Application to Five Drugs. AAPS PharmSciTech 15, 601-611. http://doi.org/10.1208/s12249-014-0084-0.

Giacoia, G.P., Taylor-Zapata, P., Zajicek, A., 2012. Eunice Kennedy Shriver National Institute of Child Health and Human Development pediatrics formulation initiative: proceedings from the second workshop on pediatric formulations. Clin Ther 34, S1-S10. https://doi.org/10.1016/j.clinthera.2012.09.013.

Gibaldi, M., Feldman, S., 1967. Establishment of sink conditions in dissolution rate determinations. Theoretical considerations and application to nondisintegrating dosage forms. J Pharm Sci 56, 1238-1242. https://doi.org/10.1002/jps.2600561005.

Goetze, O., Treier, R., Fox, M., Steingoetter, A., Fried, M., Boesiger, P., Schwizer, W., 2009. The effect of gastric secretion on gastric physiology and emptying in the fasted and fed state assessed by magnetic resonance imaging. Neurogastroenterol Motil 21, 725-e742. https://doi.org/10.1111/j.1365-2982.2009.01293.x.

Guimarães, M., Statelova, M., Holm, R., Reppas, C., Symilllides, M., Vertzoni, M., Fotaki, N., 2019. Biopharmaceutical considerations in paediatrics with a view to the evaluation of orally administered drug products–a PEARRL review. J Pharm Pharmacol 71, 603-642. https://doi.org/10.1111/jphp.12955.

Gustafsson, I., Löwdin, E., Odenholt, I., Cars, O., 2001. Pharmacokinetic and pharmacodynamic parameters for antimicrobial effects of cefotaxime and amoxicillin in an in vitro kinetic model. Antimicrob Agents Chemother 45, 2436-2440. https://doi.org/10.1128/aac.45.9.2436-2440.2001.

ICH, 2018. Biopharmaceutics Classification System based Biowaivers M9. https://www.fda.gov/media/117974/download (accessed 8 January 2019).

Löbenberg, R., Chacra, N.B., Stippler, E.S., Shah, V.P., DeStefano, A.J., Hauck, W.W., Williams, R.L., 2012. Toward global standards for comparator pharmaceutical products: case studies of amoxicillin, metronidazole, and zidovudine in the Americas. AAPS J 14, 462-472. https://doi.org/10.1208/s12248-012-9350-9.

685 Martir, J., Flanagan, T., Mann, J., Fotaki, N., 2020. Co-administration of Paediatric Medicines
686 with Food and Drinks in the Context of Their Physicochemical Properties—a Global
687 Perspective on Practices and Recommendations. AAPS J 22, 54.
688 http://doi.org/10.1208/s12248-020-0432-9.

689 Murdoch, D., Heel, R.C., 1991. Amlodipine - A review of its pharmacodynamic and
690 pharmacokinetic properties, and therapeutic use in cardiovascular disease Drugs 41, 478-505.
691 https://doi.org/10.2165/00003495-199141030-00009.

692 Paediatric Formulary Committee, 2017. BNF for Children 2016-2017. London: BMJ Group,
693 Pharmaceutical Press, and RCPCH Publications,

694 Purohit, V.S., 2012. Biopharmaceutic planning in pediatric drug development. AAPS J 14,
695 519-522. https://doi.org/10.1208/s12248-012-9364-3.

696 Reddy, N.H., Patnala, S., Löbenberg, R., Kanfer, I., 2014. In vitro dissolution of generic
697 immediate-release solid oral dosage forms containing BCS class I drugs: comparative
698 assessment of metronidazole, zidovudine, and amoxicillin versus relevant comparator
699 pharmaceutical products in South Africa and India. AAPS PharmSciTech 15, 1076-1086.
700 https://doi.org/10.1208/s12249-014-0135-6.

701 Ricci, B.M., 2013. Bridging studies in support of oral pediatric formulation development. Int J
702 Pharm 457, 323-326. https://doi.org/10.1016/j.ijpharm.2013.07.074.

703 Robinson, J., McKenzie, C., MacLeod, D., 2016. Paediatric dosing errors with oral
704 prednisolone mixture. Aust Prescr 39, 176. https://doi.org/10.18773/austprescr.2016.062.

705 Scheubel, E., Lindenberg, M., Beyssac, E., Cardot, J.-M., 2010. Small volume dissolution
706 testing as a powerful method during pharmaceutical development. Pharmaceutics 2, 351-363.
707 https://doi.org/10.3390/pharmaceutics2040351.

708 Selen, A., Cruañes, M.T., Müllertz, A., Dickinson, P.A., Cook, J.A., Polli, J.E., Kesisoglou, F.,
709 Crison, J., Johnson, K.C., Muirhead, G.T., 2010. Meeting report: applied biopharmaceutics and
710 quality by design for dissolution/release specification setting: product quality for patient
711 benefit. Springer. https://doi.org/10.1208/s12248-010-9206-0.

712 Shah, V.P., Tsong, Y., Sathe, P., Liu, J.-P., 1998. In vitro dissolution profile comparison—
713 statistics and analysis of the similarity factor, f2. Pharm res 15, 889-896.
714 https://doi.org/10.1023/A:1011976615750.

715 Shawahna, R., 2016. Pediatric biopharmaceutical classification system: using age-appropriate
716 initial gastric volume. AAPS J 18, 728-736. https://doi.org/10.1208/s12248-016-9885-2.

717 Shohin, I., Ramenskaya, G., Vasilenko, G., Malashenko, E., 2010. In vitro dissolution kinetics
718 of amlodipine tablets marketed in Russia under biowaiver conditions. Dissolut Technol 17, 20-
719 23. https://doi.org/10.14227/DT170310P20.

720 Stuart, A.V., Zuo, J., Löbenberg, R., 2014. Investigating the Dissolution Profiles of
721 Amoxicillin, Metronidazole, and Zidovudine Formulations used in Trinidad and Tobago, West
722 Indies. AAPS PharmSciTech 15, 1060-1069. https://doi.org/10.1208/s12249-014-0132-9.

723 Thambavita, D., Galappatthy, P., Mannapperuma, U., Jayakody, L., Cristofoletti, R.,
724 Abrahamsson, B., Groot, D.W., Langguth, P., Mehta, M., Parr, A., 2017. Biowaiver monograph
725 for immediate-release solid oral dosage forms: amoxicillin trihydrate. J Pharm Sci 106, 2930-
726 2945. https://doi.org/10.1016/j.xphs.2017.04.068.

727 USP, 2007. USP 30-NF 25. Rockville MD: United States Pharmacopeial Convention.

728 Van Zwieten, P., 1994. Amlodipine: an overview of its pharmacodynamic and pharmacokinetic
729 properties. Clin Cardiol 17, III3

730 Vogt, M., Derendorf, H., Krämer, J., Junginger, H., Midha, K., Shah, V., Stavchansky, S.,
731 Dressman, J., Barends, D., 2007. Biowaiver Monographs for Immediate Release Solid Oral
732 Dosage Forms: Prednisolone. J Pharm Sci 96, 27-37. https://doi.org/10.1002/jps.20817.

733 Walsh, J., Cram, A., Woertz, K., Breitkreutz, J., Winzenburg, G., Turner, R., Tuleu, C. and
734 European Formulation Initiative, 2014. Playing hide and seek with poorly tasting paediatric
735 medicines: do not forget the excipients. Adv Drug Deliv Rev 73, 14-33.
736 doi:10.1016/j.addr.2014.02.012

737 WHO, 2006. Proposal to waive in vivo bioequivalence requirements for WHO Model List of
738 Essential Medicines immediate-release, solid oral dosage forms.
739 https://www.who.int/medicines/services/expertcommittees/pharmprep/QAS04_109Rev1_Wai
740 ve_invivo_bioequiv.pdf (accessed 5 January 2019),

741 WHO, 2017. The Selection and Use of Essential Medicines: Report of the WHO Expert
742 Committee, 2017 (including the 20th WHO Model List of Essential Medicines and the 6th
743 WHO Model List of Essential Medicines for Children).
744 https://apps.who.int/iris/bitstream/handle/10665/259481/9789241210157-
745 eng.pdf?sequence=1&isAllowed=y (accessed 8 January 2019).

746 Wishart, D.S., Knox, C., Guo, A.C., Cheng, D., Shrivastava, S., Tzur, D., Gautam, B.,
747 Hassanali, M., 2007. DrugBank: a knowledgebase for drugs, drug actions and drug targets.
748 Nucleic Acids Res 36, D901-D906

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Tables

Table 1. Information of the formulations used in this study.

Drug	Formulation	Manufacturer	Dose (Lot No.)	Excipients
Amoxicillin (trihydrate)	Amoxil® capsules	GlaxoSmithKline Plc (UK)	250 mg (2398) 500 mg (X54F)	Magnesium stearate E572, gelatine, erythrosine E127, titanium dioxide E171, indigotine E132, iron oxide yellow E172 and shellac E904
	Teva® capsules	Teva Pharmaceutical Industries Ltd (UK)	250 mg (AXABV0005) 500 mg (13753)	Croscarmellose sodium, magnesium stearate, sunset yellow E110, carmosine E122, brilliant blue E133, Quinoline Yellow E104, titanium dioxide E171, methyl parahydroxybenzoate and propyl parahydroxybenzoate
	Kent® capsules	Kent Pharmaceuticals Ltd (UK)	250 mg (13095) 500 mg (15768)	Magnesium stearate, maize starch, gelatine, erythrosine E127, quinoline Yellow E104, titanium dioxide E171, red iron oxide E172
Prednisolone	Pevanti® tablets	Mercury Pharma Ltd (UK)	5 mg (17K09A) 25 mg (17F25A)	Potato starch, lactose, talc, gelatine and magnesium stearate
	Actavis® tablets	Actavis Generics Ltd (UK)	5 mg (4F46) 25 mg (YK13)	Lactose monohydrate, pregelatinized starch, sodium starch glycolate type A, iron oxide yellow E172, iron oxide red E172, glycerol dibehenate, magnesium stearate
Amlodipine (besylate)	Istin® tablets	Pfizer Ltd (UK)	5 mg (2398) 10 mg (X54F)	Calcium hydrogen phosphate anhydrous, magnesium stearate, microcrystalline cellulose and sodium starch glycolate type A.
	Sandoz® tablets	Sandoz Ltd (UK)	5 mg (HM8397) 10 mg (HK5291)	Microcrystalline cellulose, anhydrous calcium hydrogen phosphate, sodium starch glycolate type A and magnesium stearate.
	Teva® tablets	Teva Pharmaceutical Industries Ltd (UK)	5 mg (0190317) 10 mg (7940517)	Microcrystalline cellulose, calcium hydrogen phosphate, sodium starch glycolate and magnesium stearate.

Table 2. Dose unit in different age groups from early infancy through to adulthood. Paediatric reference volumes were calculated with the average weight of the age group* (Guimarães et al., 2019). Low and high drug solubility classification are denoted by red ($D_0 > 1$) and black ($D_0 < 1$) colours, respectively.

Drug	Aqueous solubility (mg/ml) (Wishart et al., 2007)	Dose strength (mg)	D_0				
			3 Years / V_0 = 54 mL	6 Years / V_0 = 79.2 mL	10 Years / V_0 = 121 mL	17 Years / V_0 = 245 mL	Adult / V_0 = 250 mL
Amoxicillin (trihydrate)	3.43	250	1.350	0.920	0.602	0.297	0.292
		500	2.699	1.841	1.205	0.595	0.583
Prednisolone	0.223	5	0.415	0.283	0.185	0.092	0.0897
		25	2.076	1.415	0.927	0.458	0.448
Amlodipine (besylate)	0.075	5	1.230	0.838	0.549	0.271	0.266
		10	2.459	1.677	1.098	0.542	0.531

(D_0 = dose unit; V_0 = gastric volume available)

*average weight: 3 years: 14.3kg; 6 years: 21kg; 10 years: 32kg; 17 years: 65kg

Table 3. f_2 similarity factor values for the comparison of drug dissolution profiles from the test and the reference formulation ($f_2 \geq 50$ denotes similarity; red values: $f_2 < 50$ denotes non similarity between profiles). (-) % drug dissolved > 85% within 15 min.

	f_2 value	**Amoxicillin - 250 mg**			**Amoxicillin - 500 mg**		
		pH 1.2	***pH 4.5***	***pH 6.8***	***pH 1.2***	***pH 4.5***	***pH 6.8***
Setup 1	Amoxil® *vs* Kent®	-	68.4	68.5	-	53.9	66.2
	Amoxil® *vs* Teva®	-	78.2	74.0	-	56.6	85.1
Setup 2	Amoxil® *vs* Kent®	-	60.6	73.1	-	55.6	63.0
	Amoxil® *vs* Teva®	-	62.8	61.0	-	63.9	62.3
Setup 3	Amoxil® *vs* Kent®	-	79.0	54.2	-	69.2	67.0
	Amoxil® *vs* Teva®	-	51.2	51.1	-	63.3	62.4
		Prednisolone - 5 mg			**Prednisolone - 25 mg**		
		pH 1.2	***pH 4.5***	***pH 6.8***	***pH 1.2***	***pH 4.5***	***pH 6.8***
Setup 1	Pevanti® *vs* Actavis®	-	-	-	-	72.4	54.0
Setup 2	Pevanti® *vs* Actavis®	-	-	-	-	**47.5**	**38.0**
Setup 3	Pevanti® *vs* Actavis®	-	-	-	83.8	73.1	81.6
		Amlodipine - 5 mg			**Amlodipine - 10 mg**		
		pH 1.2	***pH 4.5***	***pH 6.8***	***pH 1.2***	***pH 4.5***	***pH 6.8***
Setup 1	Istin® *vs* Sandoz®	-	-	65.9	-	-	**48.4**
	Istin® *vs* Teva®	-	-	58.3	-	-	50.7
Setup 2	Istin® *vs* Sandoz®	-	-	-	-	-	**40.0**
	Istin® *vs* Teva®	-	-	-	-	-	64.3
Setup 3	Istin® *vs* Sandoz®	-	-	-	-	-	81.4
	Istin® *vs* Teva®	-	-	-	-	-	52.7

Figure Captions

Figure 1. Schematic representation of the different dissolution setups tested: (1) Setup 1: 900 mL, USP II apparatus; (2) Setup 2: 250 mL, USP II apparatus; (3) Setup 3: 50 mL, mini-paddle apparatus.

Figure 1. Mean % amoxicillin dissolved (± S.D.) from Amoxil® capsules 250 mg (full lines) and 500 mg (dashed lines), in acetate buffer pH 4.5 (top) and phosphate buffer pH 6.8 (bottom), under two testing scenarios: setup 1 (USP II apparatus, 900 mL) and setup 2 (USP II apparatus, 250 mL) (left and right panels, respectively). Three agitation rates were tested: 50 rpm (blue), 75 rpm (red) and 100 rpm (black).

Figure 3. Mean % amoxicillin dissolved (± S.D.) from Amoxil®, amoxicillin Teva® and amoxicillin Kent® capsules 250 mg (left panels) and 500 mg (right panels), in SGF*sp* pH 1.2 (blue), acetate buffer pH 4.5 (green) and phosphate buffer pH 6.8 (red). Three setup scenarios were tested: (1) setup 1: 900 mL, 75 rpm, USP II apparatus; (2) setup 2: 250 mL, 75 rpm, USP II apparatus; (3) setup 3: 50 mL, 187.5 rpm, mini-paddle apparatus (from top to bottom). Dotted grey lines represent the limit for '*very rapid dissolution*' classification (> 85% dissolved within 15 min).

Figure 4. Mean % prednisolone dissolved (± S.D.) from Pevanti® and prednisolone Actavis® tablets 5 mg (left panels) and 25mg (right panels), in SGFsp pH 1.2 (blue), acetate buffer pH 4.5 (green) and phosphate buffer pH 6.8 (red). Three setup scenarios were tested: (1) setup 1: 900 mL, 50 rpm, USP II apparatus; (2) setup 2: 250 mL, 50 rpm, USP II apparatus; (3) setup 3: 50 mL, 125 rpm, mini-paddle apparatus (from top to bottom). Dotted grey lines represent the limit for '*very rapid dissolution'* classification (> 85% dissolved within 15 min).

Figure 5. Mean % amlodipine dissolved (± S.D.) from Istin®, amlodipine Sandoz® and amlodipine Teva® tablets 5 mg (left panels) and 10 mg (right panels), in SGFsp pH 1.2 (blue), acetate buffer pH 4.5 (green) and phosphate buffer pH 6.8 (red) under three testing scenarios. Three setup scenarios were tested: (1) setup 1: 900 mL, 50 rpm, USP II apparatus; (2) setup 2: 250 mL, 50 rpm, USP II apparatus; (3) setup 3: 50 mL, 125 rpm, mini-paddle apparatus (from top to bottom). Dotted grey lines represent the limit for '*very rapid dissolution'* classification (≥ 85% dissolved within 15 min).

Figure 6. Risk assessment of extending the BCS-biowaiver from the adult to the paediatric population for IR formulations of 3 model compounds. Green and red colours denote pass and fail of the rapid dissolution criterion, respectively. (N/A: not applicable; (+): meet the criterion; (-): fail the criterion; +/-: the Istin ® and Teva® products meet the criterion while the Sandoz® product does not).

Figure 1

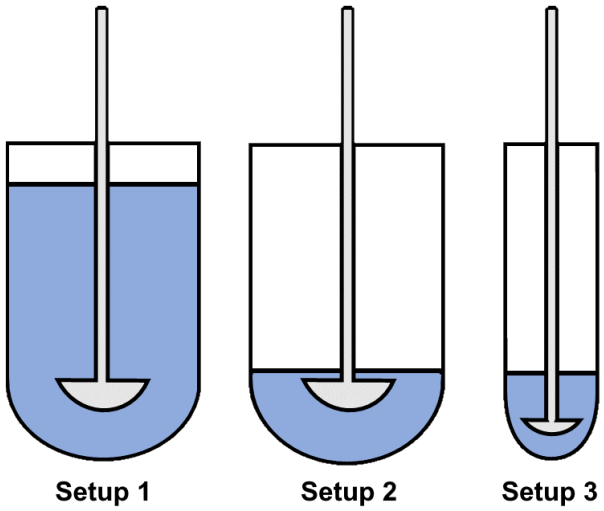


Figure 2

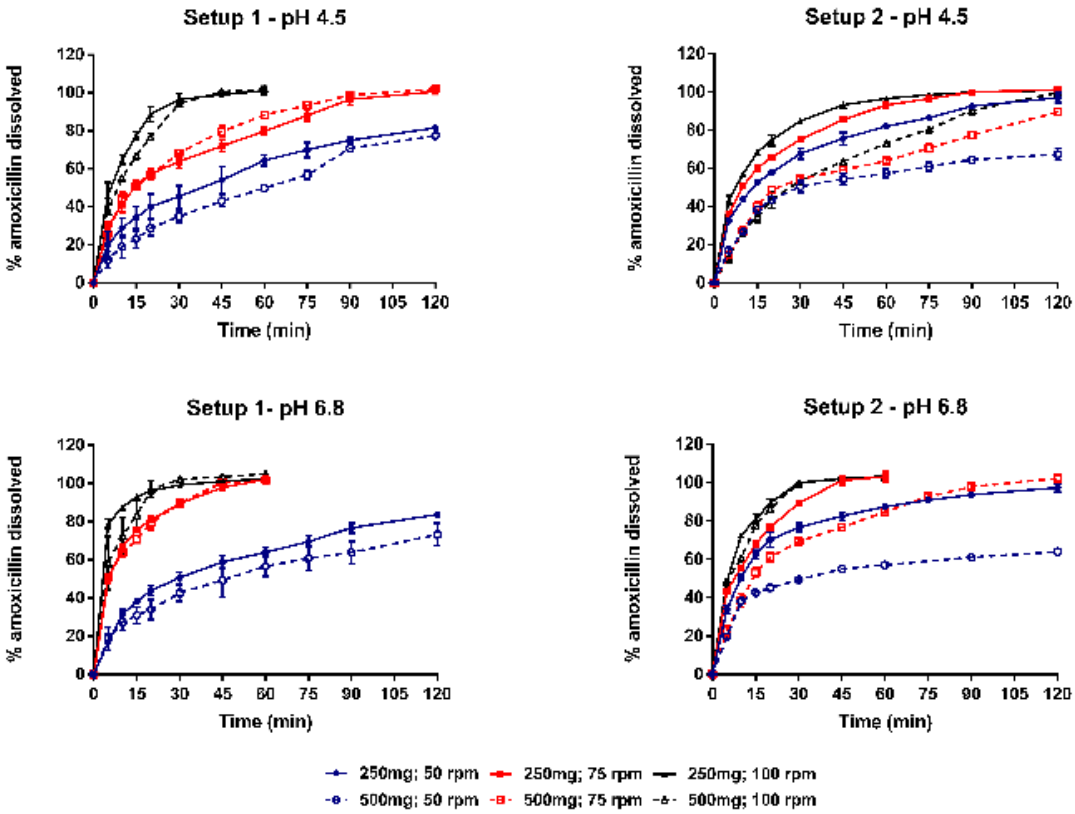

Setup 1 - pH 4.5
Setup 2 - pH 4.5
Setup 1- pH 6.8
Setup 2 - pH 6.8
% amoxicillin dissolved
Time (min)
250mg; 50 rpm
250mg; 75 rpm
250mg; 100 rpm
500mg; 50 rpm
500mg; 75 rpm
500mg; 100 rpm

Figure 3

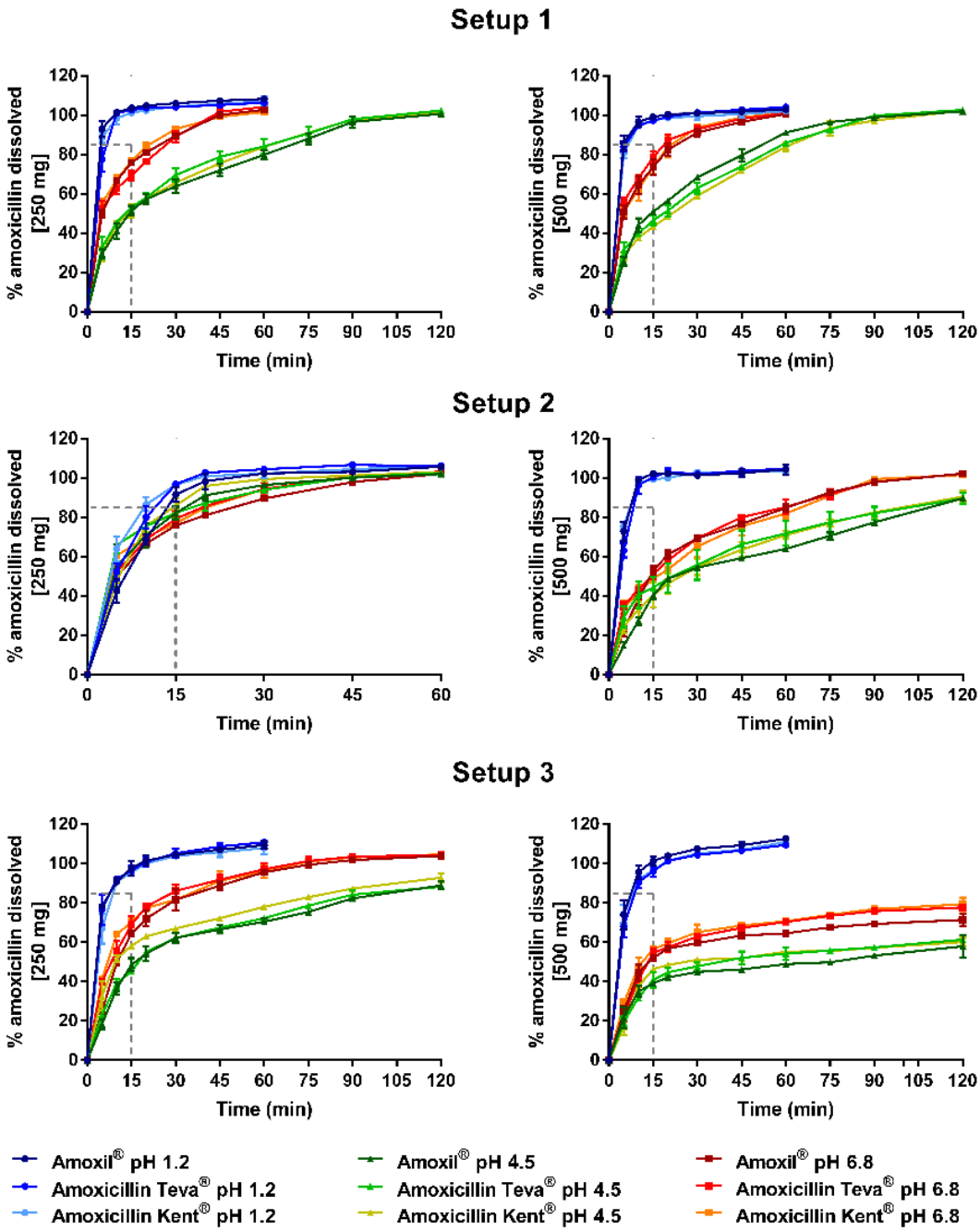
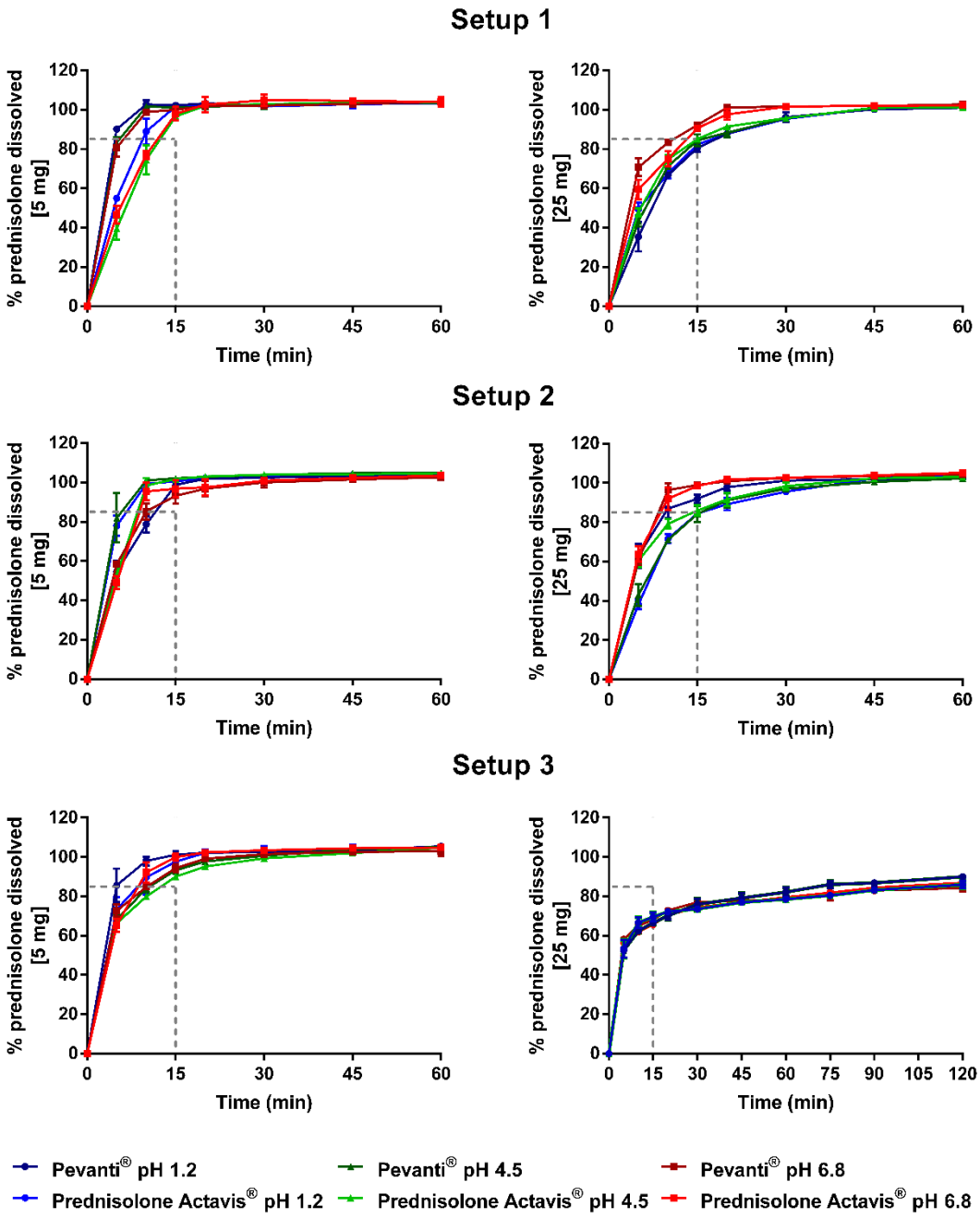
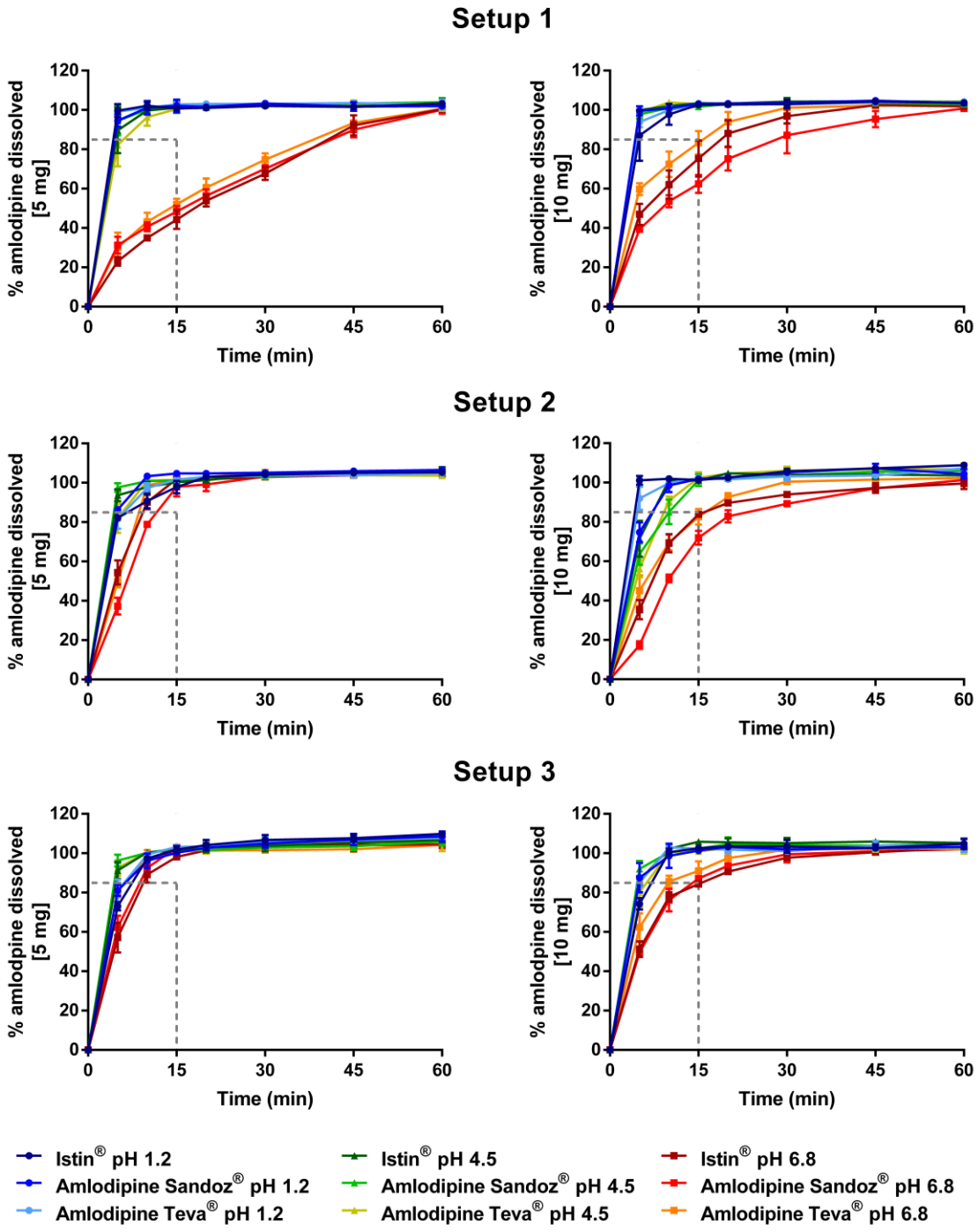

Setup 1
Setup 2
Setup 3
% amoxicillin dissolved [250 mg]
% amoxicillin dissolved [500 mg]
Time (min)
Amoxil® pH 1.2
Amoxicillin Teva® pH 1.2
Amoxicillin Kent® pH 1.2
Amoxil® pH 4.5
Amoxicillin Teva® pH 4.5
Amoxicillin Kent® pH 4.5
Amoxil® pH 6.8
Amoxicillin Teva® pH 6.8
Amoxicillin Kent® pH 6.8

Figure 4


Setup 1
Setup 2
Setup 3
% prednisolone dissolved [5 mg]
% prednisolone dissolved [25 mg]
Time (min)
Pevanti® pH 1.2
Pevanti® pH 4.5
Pevanti® pH 6.8
Prednisolone Actavis® pH 1.2
Prednisolone Actavis® pH 4.5
Prednisolone Actavis® pH 6.8

822 **Figure 5**



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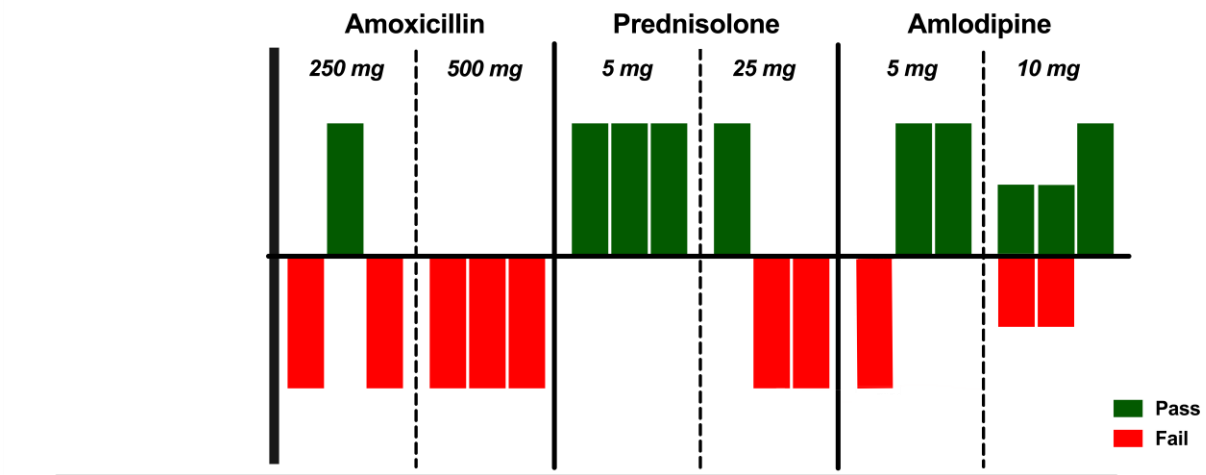
825

826 **Figure 6**



BCS Biowaiver Criteria	Setup																	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
>85% dissolved in 15 min	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	N/A
>85% dissolved in 30 min	-	+	-	-	-	-	N/A	N/A	N/A	+	+	-	-	N/A	N/A	+/-	+	+
f_2	+	+	+	+	+	+	N/A	N/A	N/A	+	-	+	+	N/A	N/A	+/-	+/-	+

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